April 4, 2003

**Background Information and Questions for Letter Review of the PFOA Preliminary Risk Assessment.**

As part of the effort by the Office of Pollution Prevention and Toxics (OPPT) to understand health and environmental issues presented by fluorochemicals in the wake of unexpected toxicological and bioaccumulation discoveries with respect to perfluorooctane sulfonates (PFOS), OPPT has been investigating perfluorooctanoic acid and its salts (PFOA). OPPT released a preliminary *Draft Hazard Assessment of Perfluorooctanoic Acid and Its Salts*, dated February 20, 2002, on March 28, 2002, and issued a minor correction to that document on April 15, 2002. That draft assessment indicated that the compound is persistent in the environment and in humans with a half life of years. The assessment noted the potential systemic toxicity and carcinogenicity, and observed that blood monitoring data suggested exposure to the general population, albeit at low levels. The Agency has since received considerable additional toxicology data that suggest a potential for developmental/reproductive toxicity and immunotoxicity, and additional human biomonitoring data that indicate low level exposures to the general population that are unexplained at this time.

On September 27, 2002, the Director of OPPT issued a memorandum announcing that OPPT would initiate a priority review to determine whether PFOA meets the criteria for action under section 4(f) of the Toxic Substances Control Act. As part of the priority review, the hazard assessment was revised and released on September 30, 2002. In addition, OPPT conducted a preliminary risk assessment of PFOA. OPPT recognizes that there are a wide range of toxicological endpoints associated with exposure to PFOA, but restricted the analysis to examine the endpoints that are included in section 4(f). These include only cancer, mutations, and birth defects. PFOA is not mutagenic so mutagenicity was not considered in the preliminary risk assessment.. In addition, PFOA is a PPARa-agonist and through this mode of action leads to the formation of liver tumors in rodents. The relevance of this mode of action for humans is currently under scientific debate, and the Agency is engaged in activities to resolve this issue. Therefore, at this time, OPPT has narrowly restricted the analysis to examine the potential risks of developmental toxicity.

OPPT welcomes comments on any aspect of this preliminary risk assessment. Expertise pertinent to the review of this assessment includes knowledge of pharmacokinetics, developmental toxicology, and risk assessment. Specifically, we would appreciate comments on the questions that follow.

Issue 1. Developmental Endpoints

The Agency's Developmental Toxicity Risk Assessment Guidelines state that the period of exposure for developmental toxicity is prior to conception to either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. Based on this definition of developmental exposure, OPPT considered developmental effects to include reductions in F1 mean pup body weight (sexes combined) on lactation days 1, 5 and 8, an increase in mortality during the first few days after weaning (both sexes), a delay in the timing of sexual maturation (both sexes), and a reduction in mean body weight postweaning (F1 males only).

Question 1.1

Please comment on the choice of developmental endpoints from the 2-generation reproductive toxicity study for this preliminary risk assessment.

Issue 2. Use of Human Biomonitoring Data

Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data. Approximately 600 individuals were involved in each of the U.S. samples of adults and children from which the serum data were derived for the general population exposures.

Question 2.1 Please comment on the adequacy of the human exposure data for use in calculating a MOE.

Issue 3. Use of Serum Data as a Measure of Internal Dose

Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data.

Question 3.1 Please comment on the use of serum data as a measure of internal dose for rats and humans for calculation of the MOE.

Issue 4. Use of F0 serum levels as an estimate of serum levels of F1 animals

The rat serum levels measured in the 2-generation reproductive toxicity study were used as an estimate of internal dose. However, serum levels were measured only in the F0 animals and only measured in the control, 10, and 30 mg/kg/day groups, not in the 1 and 3 mg/kg/day groups. The concern for developmental toxicity is for the F1 animals. The assumption has been made that the serum levels measured in the F0 animals are the best available dose estimate for the F1 animals.

Question 4.1

Please comment on the use of F0 serum values as a dose estimate for the F1 rats.

Question 4.2

Please comment on the use of serum values for F0 males as an upper estimate of peak exposure for F0 females.

Question 4.3

Recent studies suggest that three organic anion transporters (OAT), OAT1, OAT2, and OAT3, are involved in the renal transport of PFOA. A study of developmental and gender-specific influences on the expression of rat OAT in the kidney has shown that at birth all OAT mRNA levels were low. Renal OAT1 expression approaches adult level at 30 days, where at day 40 and 45 OAT1 levels were greater in males than females. OAT2 expression was minimal through day 30 but increased dramatically only in females at day 35. OAT3 expression matured the earliest and reached adult levels at 10 days. Please comment on the significance of OAT data for estimating internal dose on the postweaning rat.

**EXTERNAL PEER REVIEWERS FOR PRELIMINARY RISK ASSESSMENT OF THE DEVELOPMENTAL TOXICITY ASSOCIATED WITH EXPOSURE TO PERFLUOROOCTANOIC ACID AND ITS SALTS**

**Steve Cragg, Ph.D.**

Dr. Cragg has 25 years experience as a toxicologist working for industry with industrial chemicals and as an expert with consulting firms. During this time, he has reviewed and monitored all types and manner of toxicity studies including metabolism, reproductive toxicity and developmental toxicity studies. In addition to animal studies, Dr. Cragg has reviewed and summarized many industrial biomonitoring surveys, case studies, and human epidemiological studies. Dr Cragg summarized the complete toxicology and fate and environmental effects literature in several separate chapters for a wide variety of chemicals in "Patty's Toxicology" (5th Ed). More recently, he has written extensive reviews for the entire spectrum of health and environmental studies, as "robust summaries," under the EPA High Production Volume (HPV) Chemicals Program. This requires intimate knowledge of the procedures used to conduct such studies.

**Henry Spencer, Ph.D.**

Dr. Spencer has over 30 years of experience working as a toxicologist for the US Government. He has written and reviewed several toxicological/risk assessment documents while at the Office of Pesticides Program of the EPA. He is very familiar with the process and regulatory requirements of producing a complete toxicological document that meets the needs of EPA.

**Katherine Squibb, Ph.D.**

Dr. Squibb has over 20 years experience as a researcher and educator in the field of toxicology. She currently is the Interim Director of Program in Toxicology, Environmental Epidemiology and Toxicology at the University of Maryland School of Medicine and also works with Tetrahedron preparing IRIS documents for the HED of OPP, EPA. Dr. Squibb has also served as the President of the Metals Specialty Section of the Society of Toxicology and currently is a Councilor. She is also a Councilor for the National Capitol Area Chapter of Society of Toxicology. Her major research areas of interests are: mechanisms of trace metal metabolism and cellular toxicity and toxicity of ambient air particles. She has contributed to hundreds of technical books and journals. Dr. Squibb has also written IRIS documents for the USEPA Office of Pesticides Program.

# Peer Review of the April 4th, 2003 United States EPA Document Titled "Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and Its Salts"

**Prepared by**
**Tetrahedron, Inc.**
**April 8, 2003**

REVIEWER: Steve T. Cragg, Ph.D., DABT

## EPA Issue 1. Developmental Endpoints

The Agency's Developmental Toxicity Risk Assessment Guidelines state that the period of exposure for developmental toxicity is prior to conception to either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. Based on this definition of developmental exposure, OPPT considered developmental effects to include reductions in F1 mean pup body weight (sexes combined) on lactation days 1, 5 and 8, an increase in mortality during the first few days after weaning (both sexes), a delay in the timing of sexual maturation (both sexes), and a reduction in mean body weight postweaning (F1 males only).

### EPA Question 1.1

Please comment on the choice of developmental endpoints from the 2-generation reproductive toxicity study for this preliminary risk assessment.

### Reviewer Answer to Issue 1, Question 1.1:

The choice of endpoints from the 2-generation reproductive toxicity study used for this preliminary risk assessment are appropriate. Reductions in mean pup body weight, mortality until weaning, timing of sexual maturation, and body weight postweaning are now and have been for a long time, appropriate endpoints monitored for reproductive toxicity studies. In fact, these endpoints are described explicitly in the harmonized OECD & FIFRA testing protocol guidelines for reproductive toxicity studies.

*EPA Response:* The reviewer agrees with EPA's choice of developmental endpoints.

## EPA Issue 2. Use of Human Biomonitoring Data

Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data. Approximately 600 individuals were involved in each of the U.S. samples of adults and children from which the serum data were derived for the general population exposures.

### EPA Question 2.1

Please comment on the adequacy of the human exposure data for use in calculating a MOE.

### Reviewer Answer to Issue 2, Question 2.1:

The human data seem adequate given the large numbers of subjects monitored. The similarity in levels despite age or sex further argues that results do not reflect an atypical cohort.

*EPA Response:* The reviewer states that the human biomonitoring data are adequate.

## EPA Issue 3. Use of Serum Data as a Measure of Internal Dose

Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data.

**EPA Question 3.1**

Please comment on the use of serum data as a measure of internal dose for rats and humans for calculation of the MOE.

**Reviewer Answer to Issue 3, Question 3.1:**

Using serum data as a measure of internal dose for both rats and humans is better than using an oral dose in mg/kg-day and trying to calculate a dose in humans with similar units from a combined inhalation and dermal exposure. The latter exercise would require the use of several assumptions fraught with uncertainty and therefore requiring perhaps an overly-conservative approach. Use of serum levels "normalizes" any absorption differences that might exist between an oral exposure (in the animal study) and dermal and inhalation exposures (the routes most likely in humans), rendering moot differences in absorbed dose caused by the route of administration. Moreover, using plasma levels precludes having to account for possible differences in subsequent distribution and elimination of PFOA that might exist between rats and humans. Even metabolism differences tend to be minimized if humans and the animal model metabolize PFOA similarly. Since PFOA apparently is not metabolized to a proximate toxin but is excreted unchanged, it is reasonable to assume that the parent compound is toxic itself and that this is true in animals or humans. However, there is not confirmatory information that in humans, metabolism does not occur. All the human studies discussed in the report appear to monitor PFOA levels and not investigate possible metabolites. Thus, plasma levels of the parent PFOA are a good reflection of dose.

*EPA Response:* The reviewer agrees with the use of the serum data.

**EPA Issue 4. Use of F0 serum levels as an estimate of serum levels of F1 animals**

The rat serum levels measured in the 2-generation reproductive toxicity study were used as an estimate of internal dose. However, serum levels were measured only in the F0 animals and only measured in the control, 10, and 30 mg/kg/day groups, not in the 1 and 3 mg/kg/day groups. The concern for developmental toxicity is for F1 animals. The assumption as been made that the serum levels measured in the F0 animals are the bast available dose estimate for the F1 animals.

**EPA Question 4.1**

Please comment on the use of F0 serum values as a dose estimate for the F1 rats.

**Reviewer Answer to Issue 4, Question 4.1:**

Since F1 serum values were not collected, the F0 values must serve as surrogates. Even though exposure to the test substance was for a greater portion of their lives, it does seem reasonable to assume that a second generation would metabolize the ammonium salt of PFOA in a manner similar to their parents.

*EPA Response:* The reviewer agrees with the use of the serum data from the F0 animals.

**EPA Question 4.2**

Please comment on the use of serum values for F0 males as an upper estimate of peak exposure for F0 females.

**Reviewer Answer to Issue 4, Question 4.2:**

Using the male plasma values, which are 50 - 100 fold higher than females, profoundly increases the MOE. The fact that male serum levels are "saturated" may actually mean they are more representative of toxic dose levels particularly when serum levels were measured so long after dosing (i.e., 24 hours). The toxic manifestations of a chemical, when expressed as a plasma "dose" level, are more likely incurred at a peak level than, as in the females, when rapid excretion has reduced that level drastically. This would argue that the male levels are more representative of doses that actually caused the toxic injury.

*EPA Response:* The reviewer agrees with the approach used by EPA.

**EPA Question 4.3**

Recent studies suggest that three organic anion transporters (OAT), OAT1, OAT2, and OAT3, are involved in the renal transport of PFOA. A study of developmental and gender-specific influences on the expression of rat OAT in the kidney has shown that at birth all OAT mRNA levels were low. Renal OAT1 expression approaches adult level at 30 days, where at day 40 and 45 OAT2 levels were greater in males than females. OAT2 expression was minimal through day 30 but increased dramatically only in females at day 35. OAT3 expression matured the earliest and reached adult levels at 10 days. Please comment on the significance of OAT data for estimating internal dose on the postweaning rat.

**Reviewer Answer to Issue 4, Question 4.3:**

The authors of the report assume that OAT2, which levels are dramatically higher in female rats only, accounts for the sex difference in the more rapid excretion of PFOA by females (and consequent lower plasma levels). If true, and if OAT2 doesn't develop until approximately day 40, then young females might have been subject to higher plasma levels than after 40 days of age. This supports the argument that adult female plasma levels, being measured 24 hours after dosing and so rapidly excreted, are low estimates of rat exposure to PFOA. All this discussion of OAT notwithstanding, it seems such rapid elimination by females, coupled with sampling so long after dosing, would surely tend to underestimate dose levels. It would be important if parallels could be established in humans but such human studies have not been conducted.

*EPA Response:* The reviewer agrees that the OAT data suggest that serum PFOA levels may be higher in prepubescent female rat than in the adult rat, but the actual difference cannot be quantitated at this time.

## General Comments:

1. The reproductive toxicity study that serves as the animal extrapolation basis for this risk assessment appears to have been well conducted. Evaluation of the experimental design and monitored endpoints shows that the study satisfies the testing requirements of OPPTS 870.3800 "Reproduction and Fertility Effects," as well as the harmonized OECD Guideline for Testing of Chemicals Protocol 416 "Two-Generation Reproduction Toxicity Study."

*EPA Response:* The reviewer agrees that the two generation reproductive toxicity study was an appropriately designed and conducted study.

2. When the rat reproductive toxicity study is discussed, serum levels with units of µg/ml are used. When human survey data are discussed later in the report, units of ppm are used, without explicit definition in Tables 2 and 3 (or in the narrative), that these units are equivalent. Suggest using µg/ml (nanog/ml) or ppm (ppb) throughout the report.

*EPA Response:* The text of the preliminary risk assessment has been changed so that all serum levels are expressed as ppm or ppb.

3. In the reproductive toxicity study in rats, the authors of the report concluded that the delayed maturation seen in the F1 subjects was due to "decreased gestational age." What exactly this meant was not clearly explained in the report. If it means that the time of gestation was shorter in the 30 mg/kg-day group than in the controls, then the time between birth and sexual maturation could indeed be longer simply because the F1 rats were born less mature. If so, the delay would be explained merely because of the more immature status of the high dose rats at birth and not an effect of PFOA. If on the other hand, the authors of the report are defining "decreased gestational age" not as a result of premature birth but some sort of lessened maturity at birth due to the toxic effects of PFOA, perhaps as reflected in decreased birth weight, then this may be reaching. However, the critique by EPA of the authors statistical evaluation of this question seemed a little dismissive. What additional data would have satisfied EPA? Suggest this issue be clarified in the final draft.

*EPA Response:* The reviewer indicates that EPA has dismissed the argument that the delayed sexual maturation of the F1 pups in the two generation reproductive toxicity study is due to "decreased gestational age" for statistical reasons. The reviewer states that EPA did not provide a definition of gestational age and also indicates that EPA should state what additional data would need to be provided to support this hypothesis.

EPA has provided a sufficient discussion of this issue as well as an indication of the data that would be needed to support the hypothesis in section 3.5. As stated in the discussion, the authors of the study defined gestational age as the time in days from evidence of mating in the F0 generation until evidence of sexual maturation in the F1 generation. EPA's analysis of the data is as follows:

"While it is known and commonly accepted that changes in the body weights of offspring can affect the time to sexual maturation, whether or not gestational age, as defined by the authors, also affects the time of sexual maturation is purely speculative, especially since there were no data provided by the authors to support this relationship. Additionally, covarying gestational age with time to sexual maturation is problematic from a statistical standpoint. Since there was no significant change in the length of gestation

at 30 mg/kg/day, based on the authors' definition of 'gestational age', the decreases in gestational age would have to be due mostly to changes in time to sexual maturation. Therefore, sexual maturation is essentially being covaried with itself. Still, even if a relationship between gestational age and time to sexual maturation were shown, it merely offers an explanation for the observed delays in sexual maturation in high-dose animals, but does not diminish its significance."

4. The report is well written and generally clear in presenting the data and explaining conclusions. The following are a few specific comments that focus mostly on issues of clarity.

## Specific Comments:

Page 11, second full paragraph

Were non-ionic fluorine levels assumed to be PFOA absorbed to plasma proteins? The review of this study should so state even though other studies indicate that PFOA is not metabolized.

*EPA Response:* Yes. The text has been clarified.

Page 43, Table 2

Don't force the reader to peruse the narrative to determine the units in the table. The table should be able to "stand-alone." Also define ppm as μg/ml serum.

*EPA Response:* The text has been changed so all serum values are reported as ppm or ppb.

Page 45, Table 3

Same comment. Add footnote denoting units and define ppb as nanograms per ml serum.

*EPA Response:* The text has been changed so all serum values are reported as ppm or ppb.

Page 53, Table 6

The left column is titled "Human Serum Values" yet none appear below. It would make the table clear if this title were deleted and the corresponding title in the right column were renamed "Rat Serum Values Divided by Human Serum Values." Or, the title in the right column could simply be titled "MOEs" with a footnote defining MOE. As with the previous tables, a footnote should be adding defining ppm as micrograms per liter.

*EPA Response:* Table 6 has been clarified.

# Peer Review of the April 4th, 2003 United States EPA Document Titled "Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and Its Salts"

**Prepared by**
**Tetrahedron, Inc.**
**April 8, 2003**

**REVIEWER: Henry Spencer, Ph.D**

**QUESTIONS:**
**Issue I:**
**The Agency's Developmental Toxicity Risk Assessment Guidelines state that the period of exposure for developmental toxicity is prior to conception to either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. Based on this definition of developmental exposure, OPPT considered developmental effects to include reductions in F1 mean pup body weight (sexes combined) on lactation days 1, 5 and 8, an increase in mortality during the first few days after weaning (both sexes), a delay in the timing of sexual maturation (both sexes), and a reduction in mean body weight postweaning (F1 males only).**

**Response:**

Developmental toxicity is obviously a situation which may well have its beginning in the time frame of preconception through exposure of the parents. Exposure throughout the gestational period and weaning to the end of not only sexual maturation but to include the end of development to a mature adult animal. I personally would probably limit the definition to that and not include the entire lifespan of the organism.

*EPA Response:*

EPA has used the definition that is provided in the Agency's Developmental Toxicity Risk Assessment Guidelines.

**Question 1.1**:

**Please comment on the choice of developmental endpoints from the 2-generation reproductive toxicity study for this preliminary risk assessment.**

**Response:**

Generally, the data in the document do not provide evidence that developmental toxicity is seen in other species that does not also occur in the rat 2-generation reproduction study. The only reason that I can see that we would use the reproduction study is that the exposure that occurs there is carried thru to maturity, precohabitation, gestation, lactation, weaning and maturity again.

We see that for the most part, the effects occurring at the lowest dose are in the F1 generation and occurs in both sexes.
Lactational early weight losses generally are a consequence of milk transfer and may well stem from a high serum level of test material. NOAEL = 10 mg/kg in F1 females.

Mortality in the F1 males and females at 30 mg/kg in early days after weaning. NOAEL= 10 mg/kg
Delay in sexual maturation in F1 females and F1 males at 30 mg/kg

NOTE: This was a gavage study with very high serum spikes of test material occurring while the usual study is an oral feeding study which usually does not provide the very high serum levels in a short period of time. This type of serum level often has a detrimental effect more often than does the serum levels obtained from the feeding study.

*EPA Response:*

EPA has stated that developmental effects were noted in a prenatal developmental toxicity study in rabbits and in the two generation reproductive toxicity study in rats. Both studies were considered important for the assessment. However, only the two generation reproductive toxicity study was used in the calculation of the MOEs as serum levels were not measured in the rabbit study. Uncertainties associated with the choice of the rat study are addressed in section 5.5.

**Issue 2: Use of Human Biomonitoring Data**

**Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data. Approximately 600 individuals were involved in each of the U.S. samples of adults and children from which the serum data were derived for the general population exposures.**

**Response:**

The use of human biomonitoring data, if relevant, is probably the best that can be obtained. Because this is a preliminary risk assessment one should use the most reasonable data that can be obtained .

*EPA Response:*

Reviewer agrees with EPA approach. No comment.

**Question 2.1:**

**Please comment on the adequacy of the human exposure data for use in calculating a MOE.**

**Response:**

I believe that the data are probably not too bad if, used properly. However, the data from the blood banks with stratification to sex and age groups and covering the few cities most likely are the best that you'll have at this time.

I think that since you are producing MOEs, that the range of values should also be used in producing the MOEs in ranges.

I also believe that more data should be obtained to more thoroughly address the question of reality. Without seeing the study, I do not believe that the Streptococcal childrens data would necessarily represent anymore than one segment of the population of children, those living in possibly the lower economic levels of the District of Columbia.

*EPA Response:*

In general, the reviewer appears to agree with the use of these data. EPA has revised section 5.4 to clarify issues associated with the use of the mean and range. EPA is not clear as to the intent of the last paragraph of the reviewers comments above. However, to clarify, the sample of children included 598 children from 23 states and the District of Columbia.

**Issue 3: Use of Serum Data as a Measure of Internal Dose**

**Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data.**

**Response:**

The use of serum as a measure of internal dose is probably as good an indicator of internal dose without actually measuring the dose given. Naturally, the use of serum is only as good as the limitations that are set upon it by the test material. In this case, perfluorooctanoic acid is a reasonably good candidate to give the correct results in serum. However, one sex does not handle the chemical as well as the other and serum results must be evaluated differently for each sex. The female rat appears to handle organic acids better than the male rat with altered secretion by virtue of sex hormones with the result that the serum ½ life is very long in the male and relative short in the female.

The use of serum values in humans is plausible, but little is known of the excretion/secretion of the perfluorooctanoic acid moiety in this species. There are no data on the possible sex differences on the excretion rates of this chemical.

*EPA Response:*

In general, the reviewer agrees with the MOE approach. No comment.

**Question 3.1:**

**Please comment on the use of serum data as a measure of internal dose for rats and humans for calculation of the MOE**.

There are data on a number of human subjects with perflurooctanoic acid serum values. The data include both occupational and the general public. These data only provide a cursory insight into the possible real body burden of the chemical. Further studies will be needed to find the real activity of the chemical in the body.

The data on rats is much greater than found for humans. However, the use of the female and male data in the reproduction study must be tempered with caution. The male data appear to represent a real level of relatively high constant internal exposure. While the female serum when taken at the same time showed very low levels of chemical. Data are present in the report which indicate that with a 2 mg dose the female rat has the initial very high spike at 4.5 hours of approximately 13 ppm. By 8 hours the values are approximately 11 ppm and by 24 hours the value has dropped to the normal of approximately 0.35 ppm which is essentially the same as the value of 0.37 ppm reported in reproduction study.

It seems reasonable that there might be enough data in this additional study to be able to use it as the values for MOEs instead of using the lowest dose of 0.35 ppm. I don't see any other way to do a risk assessment with MOEs in humans without some data and human serum data is the best you have at this time.

*EPA Response:*

In general, the reviewer agrees with the use of serum levels as a measure of internal dose. The reviewer suggests that the kinetics data from the Ophaug and Singer (1980) study could be used to better delineate the serum levels in the female rat. EPA has addressed the uncertainties associated with several of the existing studies which provide kinetic and half life information in section 5.5. Generally, these studies were not used as there was a wide variation in the rat strains, method of dosing, method of measuring serum PFOA, and half life.

**Issue 4: Use of F0 serum levels as an estimate of serum levels of F1 animals**

**The rat serum levels measured in the 2-generation reproductive toxicity study were used as an estimate of internal dose. However, serum levels were measured only in the F0 animals and only measured in the control, 10, and 30 mg/kg/day groups, not in the 1 and 3 mg/kg/day groups. The concern for developmental toxicity is for the F1 animals. The**

**assumption has been made that the serum levels measured in the F0 animals are the best available dose estimate for the F1 animals.**

**Question 4.1:**

**Please comment on the use of F0 serum values as a dose estimate for the F1 rats.**

**Response:**

The serum levels measured in the 2-generation reproduction study only represent a part of the real story in the quest for an internal dose evaluation and subsequent MOEs.
We know from the data in the reports presented that: 1. PFOA is well and relatively rapidly absorbed from the stomach. 2. The report by Ophaug and Singer indicates a high spike of serum PFOA levels following 2 mg (approx. 6.6 mg/kg) which persist for at least close to 12 hours where after the level reaches the values found in the reproduction study of 0.37 ppm. 3. The reproduction study indicates that the male rat serum levels remain very high and stable for considerable time and that a value that stable would be repeatable time after time.
4. Since the value of 0.37 ppm does NOT occur immediately, and there is data to indicate that the PFOA levels stay elevated for at least one half the 24 hour time period, the 0.37 ppm value can not represent the serum internal burden for females.

*EPA Response:*

In general, the reviewer agrees with the uncertainties that EPA has addressed concerning the serum levels in the female and male rats. EPA has repeatedly stressed that the available serum data for the females in the two generation reproductive toxicity study were obtained 24 hours after dosing and therefore represent the low end of the internal dose in the female rat. EPA has also repeatedly stressed that due to the gender differences in the elimination of PFOA in the rat, the serum levels in the male rat are high due to bioaccumulation and saturation.

**Question 4.2:**

**Please comment on the use of serum values for F0 males as an upper estimate of peak exposure for F0 females.**

**Response:**

With regard to the possible use of serum values for F0 males as an upper estimate of peak exposure for F0 females it would seem inadvisable without a study or data to support it as reasonable. Since the males are treated each day by gavage and tend to not be able to transport the organic acid as does the females, the likelihood that levels would build up in the males over the peak single exposure in a 24 hour period. The females which are normally able to excrete the organic acid are able to keep the levels low. However, the data in the rat study support that the females at 30 mg/kg are showing an increasing concentration of organic acid when compared

to the females receiving only 10 mg/kg. The higher doses in the males will be even higher and shows the effects of being saturated in the transport mechanism at the 10 and 30 mg/kg doses. Since these effects are not as pronounced in the females, it is highly unlikely that the serum data of the F0 males should be surrogates for the F0 females as an upper estimate of peak exposure.

*EPA Response:*

In general, the reviewer agrees with the descriptions that EPA has provided regarding the serum levels in the male rat. EPA has stated that the serum levels in the male rats are probably much higher than the peak dose in the female rat due to bioaccumulation and saturation. EPA has also stressed that the serum levels for the F0 male rats simply represent an upper end estimate and that the peak levels in the F0 female rats are probably lower.

**Question 4.3:**

**Recent studies suggest that three organic anion transporters (OAT), OAT1, OAT2, and OAT3, are involved in the renal transport of PFOA. A study of developmental and gender-specific influences on the expression of rat OAT in the kidney has shown that at birth all OAT mRNA levels were low. Renal OAT1 expression approaches adult level at 30 days, where at day 40 and 45 OAT1 levels were greater in males than females. OAT2 expression was minimal through day 30 but increased dramatically only in females at day 35. OAT3 expression matured the earliest and reached adult levels at 10 days. Please comment on the significance of OAT data for estimating internal dose on the postweaning rat.**

**Response:**

These organic anion transporters OAT,thru OAT3 are involved with the transport of PFOA. The times frames in which they mature in the postweaning rat are significant to its well being in the study cases in the documents. As we can see the OAT3 and the OAT2 in females show expression earlier than in males while only OAT1 is at greater levels in the male than in females at the same time. This data indicates that the male is in jeopardy if he has less of an OAT level than the females taking in high levels of PFOA at an earlier age such as 10-20 days post weaning. In addition, the OAT 2 and OAT 3 transporters are the most active in the female rat. I don't believe that the OAT data are useful for estimating internal dose on the postweaning rat. Each rat will have its own complement or level of OAT and will only approximate any other rat.

*EPA Response:*

The reviewer agrees that maturation of the OATs will influence the elimination of PFOA in the prepubescent rat. However, it is not possible to quantify the impact at this time. EPA agrees and has addressed this in section 5.5.

Comments on a page-by-page basis.

PP-1 par.2-line2 It might be better to indicate the numbers of males and females in this sentence.
*EPA Response:* Correction made.

PP-1 par 2-line 9 Please indicate that there are also dogs and there appears to be a sex difference.
*EPA Response:* The dog study is described in detail in section 3.2. EPA does not agree that this study should be included in the executive summary.

PP-1 par 2- last line This gender difference has not been studied in humans.
*EPA Response:* This has been clarified in the text.

PP-1 par 3-last line Suggesting exposure from some low level source.
*EPA Response:* Interesting speculation. No comment.

PP-2 par-1-line1 The large majority of production workers----are males
*EPA Response:* This comment appears to state that proper grammar would dictate the use of the plural for males. EPA disagrees as the subject of the sentence (ie. majority) is singular not plural.
PP-2 par-5-last line Determined 24 hours after the last dosing
*EPA Response:* The details regarding the serum levels are provided in the preliminary risk assessment section of the executive summar.

PP-3 par- last The study should note it was by oral gavage, and the length of the dosing is 80 days.
*EPA Response:* These details are provided in section 3.5 and do not need to be repeated in the executive summary.

PP-8 par-2 The MP is 45- ? C. Is this 55 C?
*EPA Response*: The text was corrected.

PP-8 par- last APFO did not recently report.... Authors.....recently reported.
*EPA Response*: The text was corrected.

PP-10 par-2 Only provided 1/3 of the information. How about the other retirees?
*EPA Response*: The information was added to the text.

PP-10 par-3-line 5 It is stated that the data cannot be pooled or averaged unless the decay curves show first-order kinetics. But in the par. above the data is averaged. Wouldn't it be better to show the data in a table to give us an idea of the variation.

*EPA Response:* The text was clarified. The data are too preliminary to present in a table at this time.

PP-11 par-2 This Ophaug and Singer study certainly appears to have been overlooked or totally neglected in finding the high serum levels of the female rats at as early as 4.5 hours after a single 2 mg gavage dose.

*EPA Response:* The limitations in using the kinetic information from this study as well as the other kinetic studies were addressed in section 5.5.

PP-12 par-1 line3 At a doses of 10 - doses should be singular.
*EPA Response:* The text was corrected.

PP-21 par-2 line-2 Please state whether this hormone testing was for males or females?
*EPA Response:* The text was clarified.

PP-23 par-2 line-5 Spell the 95% or change the sentence.
*EPA Response*: The text was corrected.

PP-28 par last line-1 What was the % or grams of weight reduction?
*EPA Response:* This comment refers to a transient weight reduction in the pregnant animal. EPA does not believe that this level of detail is needed.

PP-29 par-3 line-8 After the 1 ml/kg please add "of test material"
*EPA Response:* EPA disagrees as this refers only to the dose volume, not specifically to the volume of APFO.

PP-30 par-1 Was the increase % of total pups or by % litters?
*EPA Response:* The study authors did the analysis on a per pup basis.

PP-30 par-2 In the inhalation study is it possible to put the mg/m3 into mg/kg/day?
*EPA Response:* EPA does not think this is appropriate.

PP-32 In the York study, were the litters culled or were they left to litter as they could?

*EPA Response:* The litters were not culled. The text was clarified.

PP-34 par-1 line-5 How far from the normal weighing dates were the dates of sexual maturation/ Would it make difference in the reporting of body wt. values?
*EPA Response:* The covariate analysis of the day of sexual maturation and body weight was conducted and is presented in the text.

PP-35 par-5 line-4 Treated is still misspelled.
*EPA Response*: The text was corrected.

PP-36 par-3 line-3 There is a slash mark missing after the ug .
*EPA Response:* The text was corrected.

PP-36 par-5 line-1 Is it possible to put back in the 30mg/kg/day here?
*EPA Response:* The text was clarified.

PP-39 par-5 line-3 Was there data available to indicate the sex of the pups found dead with no milk in their stomachs?
*EPA Response:* EPA does not agree that this information is necessary.

PP-40 par-2 line-1 Were the increases in dead pups on a litter basis?
*EPA Response:* Yes, the prenatal and lactational deaths were analyzed on a litter basis by the study authors, as well as by EPA statisticians.

PP-46 par-2 What were the ages that were included in calculations for child-bearing? If so, then the data from the different blood banks and programs should be culled to note that and use only that data. Please not that this has happened here. We didn't use the 2-8 year females in the . or the 69 year females in the calculations.
*EPA Response:* EPA had gender specific data for the geometric mean and range, but not for the arithmetic mean. Since the geometric means for the males and females were very similar (4.2 ppb for females and 4.9 ppb for males), the value used in the MOE calculation was the mean for sexes combined which was 4.6 ppb. EPA does not think that this significantly impacts the calculations.

PP-49 par-11ine-14 At 10 mg/kg don't the data indicate that the body wts started before day 36 postweaning?
*EPA Response:* The text was clarified.

PP-49 par-1 line-16 Please explain this sentence "No treatment related effects were observed at any doses test in the study."?
*EPA Response:* This sentence was referring to the F2 pups.

PP-55 par-4 line-10 Is the 10 mg/kg correct or is it 2 mg/kg?
*EPA Response:* Actually they administered a total dose of 2 mg to the female rats that weighed 250 g. This is actually a dose of 8 mg/kg. The text was corrected.

A General comment for the document is that it is fairly complete. I'm not sure just why the writer appears to be not aware of a piece of data that would give a better estimate of a high value and shows the decrease in serum F over a period of almost 24 hours which gives the almost identical value at that point as the one in the rat reproductive toxicity study.

*EPA Response:* This comment has been addressed above.

Please allow the reader to see the real data numbers interspersed in the individual study write-ups. This would give a better support to the statements such as statistically significantly increased or decreased.

*EPA Response:* Where appropriate, these details have been provided.

# Peer Review of the April 4th, 2003 United States EPA Document Titled "Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and Its Salts"

**Prepared by**
**Tetrahedron, Inc.**
**April 8, 2003**

**REVIEWER:Katherine Squibb, Ph.D.**

## INTRODUCTION

On April 4, 2003, the U.S. EPA's Office of Pollution, Prevention, and Toxics released a preliminary risk assessment document on perfluorooctanoic acid and its salts. As part of the review process, the U.S. EPA requested comments addressing four separate issues evaluated in the preliminary risk assessment. Tetrahedron, Inc. has carefully reviewed the preliminary risk assessment, and has generated responses to each of the issues requiring comment. Responses to each issue appear below.

## RESPONSES

**Issue 1: Developmental Endpoints**

**The Agency's Developmental Toxicity Risk Assessment Guidelines state that the period of exposure for developmental toxicity is prior to conception to either parent, through prenatal development and continuing until sexual maturation. In contrast, the period during which a developmental effect may be manifested includes the entire lifespan of the organism. Based on this definition of developmental exposure, OPPT considered developmental effects to include reductions in F1 mean pup body weight (sexes combined) on lactation days 1, 5 and 8, an increase in mortality during the first few days after weaning (both sexes), a delay in the timing of sexual maturation (both sexes), and a reduction in mean body weight postweaning (F1 males only).**

**U.S. EPA question 1.1 requested comment on the choice of developmental endpoints from the two-generation reproductive toxicity study that served as the basis of the preliminary risk assessment.**

Tetrahedron concurs with the U.S. EPA's decision to base the preliminary risk assessment of perfluorooctanoic acid (PFOA) upon developmental effects reported in the York (2002) two-generation study. Among the various types of toxicity studies presented in the U.S. EPA's April 4, 2003 preliminary risk assessment and November 4, 2002 draft hazard assessment of perfluorooctanoic acid (U.S. EPA 2002, 2003), developmental effects appear to be the most sensitive critical effect among those examined to date. The developmental effects reported in the York study, including significant decreases in body weights, body weight gains, terminal body weights, and delays in sexual maturation among F1 generation offspring, are relevant endpoints

to be considered when assessing potential developmental health risks (U.S. EPA 1991). Although the delays in sexual maturation reported among high-dose (30 mg/kg/day) F1 generation offspring may be partially explained by differences in gestational age or body weights, such explanations cannot completely account for the occurrence of this developmental effect, and fail to discount its biological significance. Developmental effects reported at lower dose levels among F1 generation offspring (*e.g.*, decreased body weights) are also considered biologically significant, and as such, result in the assignment of low NOAELs and LOAELs compared to other toxicity studies performed on PFOA. In the absence of additional data to explain the occurrence of developmental effects occurring at these low dose levels, their biological significance should not be discounted.

*EPA Response:* The reviewer agrees with the use of the endpoints from the two generation reproductive toxicity study. However, it should be noted that EPA chose these endpoints because this preliminary assessment focuses only on developmental toxicity; these endpoints were not necessarily the "critical" endpoints in the entire toxicological data base.

**Issue 2: Use of Human Biomonitoring Data**

**Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data. Approximately 600 individuals were involved in each of the U.S. samples of adults and children from which the serum data were derived for the general population exposures.**

**U.S. EPA question 2.1 requested comment on the adequacy of the human exposure data for use in calculating margins of exposure in adults and children.**

Human exposure data used by U.S. EPA are considered inadequate for MOE derivation. The PFOA serum levels sampled from blood donated by 645 adults (age 20-69 years) are derived from males and females. Ideally, the mean PFOA serum concentration used in the MOE estimation for adults should have been calculated entirely from blood samples collected from females since women of child-bearing age are considered the potential population at risk. It should be noted, however, that adult males in this sample had higher serum PFOA levels than females, so inclusion of serum PFOA concentration data from males most likely resulted in a

lower MOE estimation, and likely overestimated potential health risks from PFOA exposure.[1] It is not clear why U.S. EPA used the mean PFOA concentration calculated from 645 male and female blood samples instead of using a mean PFOA concentration calculated from only 313 female blood samples from this data set. Secondly, it is impossible to determine whether this data set is an accurate representation of the American population. Such a criticism also applies to the 598 blood samples collected from 2-12 year old children, although the data set from which the children's blood was sampled appears to be fairly comprehensive (23 states plus the District of Columbia). Although the MOE estimation includes adequate caveats, including a discussion of data weaknesses, it is recommended that additional resources be devoted to improving the estimates of average serum PFOA concentrations, particularly in adults.

*EPA Response:* For the adults, EPA had gender specific data for the geometric mean and range, but not for the arithmetic mean. Since the geometric means for the males and females were very similar (4.2 ppb for females and 4.9 ppb for males), the value used in the MOE calculation was the mean for sexes combined which was 4.6 ppb. EPA does not think that this significantly impacts the calculations. With reference to the issue of whether the samples are representative of the US population, it should be noted that the adult serum samples came from several cities including Los Angeles, Minneapolis, Charlotte, Boston, Portland, Oregon, and Hagerstown, Maryland. The children's serum samples came from 23 states and the District of Columbia. These samples were not from areas in the vicinity of manufacturing facilities. EPA believes that this is a fairly wide sampling of the US population, and is appropriate to use at this time. In reference to the footnote below, EPA has corrected the text.

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[1]It appears as if there is a typographical error on page 46 relating to the mean serum PFOA concentrations in blood samples collected from 645 adult males and females. The U.S. EPA preliminary risk assessment document identifies the geometric mean of PFOA in male and female adults as 37.8 ppb and 32.1 ppb, respectively. How can these sex-specific concentrations be so high when the preliminary risk assessment document states (on page 45) that the combined geometric mean of serum PFOA in male and female adults from this same population is only 4.6 ppb?

**Issue 3: Use of Serum Data as a Measure of Internal Dose**

**Margins of exposure (MOE) in this preliminary risk assessment were calculated using serum levels from the rat two-generation reproductive study and human serum levels obtained from biomonitoring data.**

**U.S. EPA question 3.1 requested comment on the use of serum data as a measure of internal PFOA dose for rats and humans. These data were used in calculation of the MOE.**

In theory, the use of serum data as a measure of internal dose is a viable method in which to approximate internal doses of PFOA in both rats and humans. Following oral and inhalation exposures, PFOA distributes to plasma, in addition to major organs such as the liver and kidney. In practice, however, use of serum data is associated with numerous shortcomings that significantly reduces the accuracy of internal dose estimations. Pharmacokinetic studies in rats have failed to consistently demonstrate a significant correlation between administered PFOA dose and serum PFOA concentration (*e.g.*, Ylinen et al. 1990). This may be due in part to saturation of plasma proteins at PFOA concentrations equal to or greater than 30 mg/kg/day. In addition, animals administered repeat doses of PFOA (*e.g.*, Ylinen et al. 1990) have demonstrated that there are gender differences in serum PFOA concentrations that should be taken into account when evaluating uncertainties associated with the MOE estimation. Because of these shortcomings, serum data in rats should only be considered extremely rough approximations of internal dose.

*EPA Response:* The reviewer agrees with the use of the data, and the caveats that EPA has provided. With reference to the issue regarding the correlation of the administered dose and the serum levels, this relationship is linear in male rats, but saturation had occurred at the time the serum samples were measured in many of the studies.

**Issue 4: Use of F0 serum levels as an estimate of serum levels of F1 animals**

**The rat serum levels measured in the 2-generation reproductive toxicity study were used as an estimate of internal dose. However, serum levels were measured only in the F0 animals and only measured in the control, 10, and 30 mg/kg/day groups, not in the 1 and 3 mg/kg/day groups. The concern for developmental toxicity is for the F1 animals. The assumption has been made that the serum levels measured in the F0 animals are the best available dose estimate for the F1 animals.**

**U.S. EPA question 4.1 requested comment on the use of F0 serum levels as internal dose estimates among F1 animals.**

Use of F0 serum levels to estimate internal doses among F1 animals from the York (2002) two-generation study introduces a false sense of certainty in the MOE estimates. Instead of estimating serum levels of F1 rats based upon from F0 serum levels, it would be much better for U.S. EPA to contact York and determine whether blood samples from the 1 and 3 mg/kg/day dose groups were frozen as part of the archival process, and have the archived F1 blood samples analyzed to determine actual serum PFOA concentrations. The U.S. EPA preliminary risk assessment does not explain why serum PFOA measurements were only conducted on F0 rats, and also fails to explain why PFOA measurements were only conducted among rats from the 0, 10, and 30 mg/kg/day dose groups. The preliminary risk assessment identifies numerous shortcomings associated with use of F0 serum data that cannot be addressed through an evaluation of PFOA's pharmacokinetics. These shortcomings include (1) uncertainties associated with estimating serum PFOA levels in blood sampled 24 hours post-dosing; and (2) uncertainties associated with estimating serum PFOA levels among 3 mg/kg/day F1 rats based upon serum PFOA levels among 10 mg/kg/day F0 rats. Uncertainties associated with issue (1) primarily affect the estimation of serum PFOA levels among F1 female rats. Serum data in F0 rats are based upon blood samples collected 24 hours following the last dose. The serum half-life of PFOA in female rats is less than 24 hours. Therefore, serum PFOA concentrations in female rats do not represent peak PFOA serum concentrations, resulting in an underestimation of the internal PFOA dose. The risk assessment attempts to account for this underestimation by stating that peak serum PFOA levels in females are likely to be lower than measured in male rats due to differences in elimination rates. While this may be true, the statement is speculative in nature, and introduces additional uncertainty into the MOE estimate. As a consequence of issue (1), MOE estimates will be too low for females, and may overstate the potential health risk. Uncertainties associated with issue (2) may have a profound effect on the MOE estimate in light of the nonlinear relationship between PFOA dose and PFOA serum concentration. Use of serum PFOA levels from 10 mg/kg/day F0 rats overestimates PFOA serum concentrations in 3 mg/kg/day F1 rats, and results in MOE estimates that are too high, in effect understating the potential health risk. The magnitude of uncertainty associated with the MOE estimates is

evidenced by the wide range of MOE values (88-11,109). Due to these shortcomings, the EPA should to determine whether archived blood samples can be obtained from the 1 and 3 mg/kg/day dose groups in order to measure serum PFOA concentrations. This would improve the MOE estimates and will ensure that the MOEs are not underestimated.

*EPA Response:* EPA has already contacted the sponsor of the study to determine whether serum samples had been collected for the F1 animals or for other dose groups. There are no archived samples to analyze. In lieu of this information, the reviewer agrees with the approach EPA has used for the preliminary risk assessment. In addition, EPA has addressed the uncertainties raised by the reviewer.

**U.S. EPA question 4.2 requested comment on the use of serum values for F0 males as an upper estimate of peak exposure for F0 females.**

There are sufficient uncertainty underlying differences between male and female PFOA serum concentrations in repeat-dose rat studies to justify using serum values for F0 males to provide upper estimates of peak exposure for F0 females. The Ylinen et al. (1990) repeat-dose study does indicate that male rats administered repeated doses of 3, 10, and 30 mg/kg/day PFOA have consistently higher PFOA serum concentrations than females at all three dose levels. This provides some assurance that F0 male serum values are higher than F0 females. However, without additional pharmacokinetic data, it is extremely difficult to know whether serum values in F0 males are entirely accurate predictors of peak serum values in F0 females. There are apparently no absorption studies that have examined differences between the rate and/or percent of absorption in males verses females.

*EPA Response:* All kinetic studies show that serum values in the male rat are higher than in the female rat due to the gender differences in elimination. EPA has therefore appropriately presented the serum values for the F0 males as representing a high end estimate of the peak values in the F0 females. The uncertainties associated with this approach have been addressed in section 5.5.

**U.S. EPA question 4.3 requested comment on the significance of OAT data for estimating internal doses among postweanling rats.**

Pharmacokinetic data presented in the preliminary risk assessment provide evidence to suggest that OAT data may be more significant for estimating internal doses of PFOA among female postweanling rats than male postweanling rats. Three rat studies cited in the preliminary risk assessment provide data suggesting that female rats excrete PFOA or AFPO to a much greater extent than male rats via an active secretion process. Specifically, Hanhijarvi et al. (1982) investigated the effect of probenecid administration among male and female Holtzmann rats. Probenecid strongly inhibits active renal secretion of organic acids. Hanhijarvi et al. reported that intraperitoneal injection of probenecid was not found to significantly influence the excretion of APFO among male rats, while in female rats, probenecid had a significant affect on APFO excretion. As part of a study in rats designed to examine the effects of adrogens and estrogens upon PFOA excretion, Vanden Heuvel et al. (1992a) administered probenecid (dose not identified) to male and female rats (strain not identified). They found that probenecid decreased the high rate of renal PFOA excretion among castrated male rats but had no effect on male rats with intact testis. Kudo et al. (2002) also investigated the effect of sex hormones upon PFOA excretion in male and female rats (strain not identified), as well as mRNA levels of organic anion transporters (OAT). Kudo et al. treated rats with probenecid (dose not identified), and found that renal clearance of PFOA was "markedly reduced" among male rats, castrated male rats, and female rats. Kudo et al. determined that the level of OAT2 mRNA in male rats was only 13% that in female rats, and concluded that OAT2 and OAT3 are responsible for urinary elimination of PFOA in the rat. They found that castration or estradiol treatment in male rats increased the level of OAT2 mRNA whereas treatment of castrated male rats with testosterone reduced it. Ovariectomy of female rats significantly increased the level of OAT3 mRNA. Collectively, these three studies demonstrate that female rats utilize active organic anion transporters to a much greater degree than male rats. Therefore, estimating internal PFOA doses based on OAT mRNA expression is not likely to be useful for male rats. Moreover, an accurate estimation of internal PFOA doses in female postweanling rats is likely to be precluded by the fact that expression of the primary OAT transporter in female rats (OAT2) is not mature until day 35 in females. It would be difficult to estimate the internal dose in postweanling female rats (*i.e.*, those rats older

than 22 days) in light of the fact that the primary OAT transporter is not mature until at least day 35.

*EPA Response:* The reviewer agrees that maturation of the OATs will influence the elimination of PFOA in the prepubescent rat. However, it is not possible to quantify the impact at this time. EPA agrees and has addressed this in section 5.5.

**General Comments Relating to the Preliminary Risk Assessment**

**Adequacy of metabolism and pharmacokinetic study descriptions:** Many of the studies described in Section 3.2 are missing important details that would assist in the evaluation of each study. For example, the specific strain of animal and specific numbers of animals evaluated are left out of the study summaries.

*EPA Response:* This information was added to the text.

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